Appendix A - Clean version



SEQUENCE LISTING

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<110> Sera, Takashi
<120> Zinc Finger Domain Recognition Code and Uses Thereof
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      2001-07-23
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      US 60/220,060
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       (1)..(32)
      Amino acids 1-3, 10-21 and 29-32 are Xaa wherein Xaa = any
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 amino acid.
<220>
      VARIANT
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      (5)..(8)
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<223> Amino acids 5-8 are Xaa wherein Xaa = any amino acid, and up
 to two can be missing.
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      (23)..(27)
<223> Amino acids 23-27 are Xaa wherein Xaa = any amino acid, and up
 to two can be missing.
<400> 1
Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa
                                     10
                                                         15
                5
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Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Xaa His Xaa Xaa Xaa
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      Zinc finger domain
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= any
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      (5)..(8)
<223> Amino acids 5-8 are Xaa wherein Xaa = any amino acid, and up
 to two can be missing.
<220>
<221> VARIANT
<222> (23)..(27)
<223> Amino acids 23-27 are Xaa wherein Xaa = any amino acid, and up
 to two can be missing.
<220>
<221> VARIANT
<222> (15)..(15)
<223> Amino acid 15 is Xaa wherein Xaa = Z-1 wherein Z-1 = Arg or Lys,
      Gln or Asn, Thr, Met, Leu or Ile, or Glu or Asp.
<220>
<221> VARIANT
<222> (17)..(17)
<223> Amino acid 17 is Xaa wherein Xaa = Z2 wherein Z2 = Ser or Arg,
 Asn, Gln, Thr, Val or Ala, or Asp or Glu.
<220>
<221> VARIANT
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<220>

<221> VARIANT

<222> (18)..(18)

<223> Amino acid 18 is Xaa wherein Xaa = Z3 wherein Z3 = His or Lys,

Asn or Gln, Ser, Ala or Met, or Asp or Glu.

<222> (21)..(21)...:

<223> Amino acid 21 is Xaa wherein Xaa = Z6 wherein Z6 = Arg or Lys,
Gln or Asn, Thr, Tyr, Leu, Ile or Met, or Glu or Asp.

<400> 2

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa 1 1 10 15

<210> 3

<211> 196

<212> PRT

<213> Artificial Sequence

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1 5 10 15

Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met 65 70 75 80

Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys

Asp Gly Gly Ser Gly Lys Lys Gln His Ile Cys His Ile Gln 100 105 110

Gly Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu 115 120 125

Arg Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys 130 135 140

Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr 145 150 155 160

His Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe 165 170 175

Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys
180 185 190

Lys Gly Gly Ser 195

<210> 4

<211> 99

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Val Pro Ile Pro Gly Lys Lys Gln His Ile Cys His Ile Gln Gly
1 5 10 15

Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg 20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly 35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His 50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met 65 70 75 80

Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys 85 90 95

Gly Gly Ser

<210> 5

<211> 99

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Met Glu Lys Leu Arg Asn Gly Ser Gly Asp Pro Gly Lys Lys Lys Gln
1 10 15

His Ala Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asn Leu 20 25 30

Gln Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
35 40 45

Glu Cys Gly Lys Ser Phe Ser Arg Ser Ser His Leu Gln Gln His Gln 50 55 60

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys 65 70 75 80

Ser Phe Ser Arg Ser Asp His Leu Ser Arg His Gln Arg Thr His Gln 85 90 95

Asn Lys Lys

<210> 6

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His Ala Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asn Leu 20 25 30

Gln Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro 35 40 45

Glu Cys Gly Lys Ser Phe Ser Glu Ser Ser Asp Leu Gln Arg His Gln 50 55 60

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys 65 70 75 80

Ser Phe Ser Arg Ser Asp His Leu Ser Arg His Gln Arg Thr His Gln 85 90 95

<210> 7

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<212> PRT

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<223> Zinc finger protein

<400> 7

Met Glu Lys Leu Arg Asn Gly Ser Gly Asp Pro Gly Lys Lys Gln 1 10 15

His Ala Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asn Leu 20 25 30

Gln Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro 35 40 45

Glu Cys Gly Lys Ser Phe Ser Arg Ser Ser His Leu Gln Glu His Gln 50 55 60

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys

70

75

80

Ser Phe Ser Arg Ser Asp His Leu Ser Arg His Gln Arg Thr His Gln 85 90 95

Asn Lys Lys

<210> 8

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Met Glu Lys Leu Arg Asn Gly Ser Gly Asp Pro Gly Lys Lys Gln
5 10 15

His Ala Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asn Leu 20 25 30

Gln Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro 35 40 45

Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asn Leu Gln Arg His Gln 50 55 60

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys 65 70 75 80

Ser Phe Ser Arg Ser Asp His Leu Ser Arg His Gln Arg Thr His Gln 85 90 95

Asn Lys Lys

<210> 9

<211> 99

<212> PRT

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Met Glu Lys Leu Arg Asn Gly Ser Gly Asp Pro Gly Lys Lys Lys Gln 1 5 10 15

His Ala Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asn Leu 20 25 30

Gln Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro 35 40 45

Glu Cys Gly Lys Ser Phe Ser Arg Ser Ser Asn Leu Gln Glu His Gln 50 55 60

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys 65 70 75 80

Ser Phe Ser Arg Ser Asp His Leu Ser Arg His Gln Arg Thr His Gln 85 90 95

Asn Lys Lys

<210> 10

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1 5 10 15

His Ala Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asn Leu 20 25 30

Gln Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro 35 40 45

Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asp Leu Gln Arg His Gln 50 55 60

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys 65 70 75 80

Ser Phe Ser Arg Ser Asp His Leu Ser Arg His Gln Arg Thr His Gln 85 90 95

Asn Lys Lys

<210> 11

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<213> Human

<400> 11

Met Arg Leu Ala Lys Pro Lys Ala Gly Ile Ser Arg Ser Ser Ser Gln
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Gly Lys Ala Tyr Glu Asn Lys Arg Lys Thr Gly Arg Gln Arg Glu Lys
20 25 30

Trp Gly Met Thr Ile Arg Phe Asp Ser Ser Phe Ser Arg Leu Arg Arg

Ser Leu Asp Asp Lys Pro Tyr Lys Cys Thr Glu Cys Glu Lys Ser Phe 50 55 60

Ser Gln Ser Ser Thr Leu Phe Gln His Gln Lys Ile His Thr Gly Lys 65 70 75 80

Lys Ser His Lys Cys Ala Asp Cys Gly Lys Ser Phe Phe Gln Ser Ser 85 90 95

Asn Leu Ile Gln His Arg Arg Ile His Thr Gly Glu Lys Pro Tyr Lys 100 105 110

Cys Asp Glu Cys Gly Glu Ser Phe Lys Gln Ser Ser Asn Leu Ile Gln 115 , 120 125

His Gln Arg Ile His Thr Gly Glu Lys Pro Tyr Gln Cys Asp Glu Cys 130 135 140

Gly Arg Cys Phe Ser Gln Ser Ser His Leu Ile Gln His Gln Arg Thr .

145 150 155 160

His Thr Gly Glu Lys Pro Tyr Gln Cys Ser Glu Cys Gly Lys Cys Phe 165 170 175

Ser Gln Ser Ser His Leu Arg Gln His Met Lys Val His Lys Glu Glu 180 185 190

Lys Pro Arg Lys Thr Arg Gly Lys Asn Ile Arg Val Lys Thr His Leu 195 200 205

Pro Ser Trp Lys Ala Gly Thr Glu Gly Ser Leu Trp Leu Val Ser Val 210 215 220

Lys Tyr Arg Ala Phe 225

<210> 12

<211> 393

<212> PRT

<213> Mouse

<400> 12

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1 10 15

Glu Ala Phe Glu Ser Gly Asp Gln Ala Glu Arg Pro Trp Gly Asp Leu 20 25 30

Thr Ala Glu Glu Trp Val Ser Tyr Pro Leu Gln Gln Val Thr Asp Leu 35 40 45

Leu	Val 50	His	Lys	Gľú	Ala	His 55	Ala	Gly	Ile	Arg	Tyr 60	His	Ile	Cys	Ser
Gln 65	Cys	Gly	Lys	Ala	Phe 70	Ser	Gln	Ile	Ser	Asp 75	Leu	Asn	Arg	His	Gln 80
Lys	Thr	His	Thr	Gly 85	Asp	Arg	Pro	Tyr	Lys 90	Cys	Tyr	Glu	Cys	Gly 95	Lys
Gly	Phe	Ser	Arg 100	Ser	Ser	His	Leu	Ile 105	Gln	His	Gln	Arg	Thr 110	His	Thr
Gly	Glu	Arg 115	Pro	Tyr	Asp	Cys	Asn 120	Glu	Cys	Gly	Lys	Ser 125	Phe	Gly	Arg
Ser	Ser 130	His	Leu	Ile	Gln	His 135	Gln	Thr	Ile	His	Thr 140	Gly	Glu	Lys	Pro
His 145	Lys	Cys	Thr	Glu	Cys 150	Ala	Lys	Ala	Ser	Ala 155	Ala	Ser	Pro	His	Leu 160
Ile	Gln	His	Gln	Arg 165	Thr	His	Ser	Gly	Glu 170	Lys	Pro	Tyr	Glu	Cys 175	Glu
Glu	Cys	Gly	Lys 180	Ser	Phe	Ser	Arg	Ser 185	Ser	His	Leu	Ala	Gln 190	His	Gln
Arg	Thr	His 195	Thr	Gly	Glu	Lys	Pro 200	Tyr	Glu	Cys	His	Glu 205	Cys	Gly	Arg
Gly	Phe 210	Ser	Glu	Arg	Ser	Asp 215	Leu	Ile	Lys	His	Tyr 220	Arg	Val	His	Thr
Gly 225	Glu	Arg	Pro	Tyr	Lys 230	Cys	Asp	Glu	Cys	Gly 235	Lys	Asn	Phe	Ser	Gln 240
Asn	Ser	Asp	Leu	Val 245	Arg	His	Arg	Arg	Ala 250	His	Thr	Gly	Glu	Lys 255	Pro
Tyr	His	Cys	Asn 260	Glu	Cys	Gly	Glu	Asn 265	Phe	Ser	Arg	Ile	Ser 270	His	Leu
Val	Gln	His 275	Gln	Arg	Thr	His	Thr 280	Gly	Glu	Lys	Pro	Tyr 285	Glu	Cys	Thr
Ala	Cys 290	Gly	Lys	Ser	Phe	Ser 295	Arg	Ser	Ser	His	Leu 300	Ile	Thr	His	Gln
Lys 305	Ile	His	Thr	Gly	Glu 310	Lys	Pro	Tyr	Glu	Cys 315	Asn	Glu	Cys	Trp	Arg 320

Ser Phe Gly Glu Arg Ser Asp Leu Ile Lys His Gln Arg Thr His Thr 325 330 Gly Glu Lys Pro Tyr Glu Cys Val Gln Cys Gly Lys Gly Phe Thr Gln 345 Ser Ser Asn Leu Ile Thr His Gln Arg Val His Thr Gly Glu Lys Pro 360 Tyr Glu Cys Thr Glu Cys Asp Lys Ser Phe Ser Arg Ser Ser Ala Leu 370 375 Ile Lys His Lys Arg Val His Thr Asp 390 <210> 13 <211> 28 <212> PRT <213> Artificial Sequence <220> <223> Zinc finger domain. <220> <221> VARIANT <222> (13)..(13) <223> Amino acid 13 is Xaa wherein Xaa = Z-1 wherein Z-1 = Arg or Lys, Gln or Asn, Thr, Met, Leu or Ile, or Glu or Asp. <220> <221> VARIANT <222> (15)..(15) <223> Amino acid 15 is Xaa wherein Xaa = Z2 wherein Z2 = Ser or Arg, Asn or Gln, Thr, Met, or Ala, or Asp or Glu. <220> <221> VARIANT (16)..(16) <222> <223> Amino acid 16 is Xaa wherein Xaa = Z3 wherein Z3 = His or Lys, Asn or Gln, Ser, Ala, or Met, or Asp or Glu. <220> <221> VARIANT <222> (19)..(19)<223> Amino acid 19 is Xaa wherein Xaa = Z6 wherein Z6 = Arg or Lys, Gln or Asn, Thr, Tyr, Leu, Ile or Met, or Glu or Asp.

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Leu Gln Xaa His Gln Arg Thr His Thr Gly Glu Lys
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      16
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       28
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Leu Gln Gln His Gln Arg Thr His Thr Gly Glu Lys
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<210>

<211>

17

28

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Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg
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                                    10
Leu Glu Trp Glu Leu Gln Ala Leu Glu Lys Glu Leu Ala Gln
                                25
            20
<210>
       20
<211>
       30
<212>
       PRT
<213>
      Artificial Sequence
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       Basic dimerization peptide
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20

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	24							
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		<i>f.</i>						
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       ZFP target sequence
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       ZFP target sequence
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tatatataag taaggtacta tatata
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       Amino acid 15 is "Xaa" wherein "Xaa" = is any amino acid.
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Leu Gln Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
                                                     30
            20
                                 25
Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Asn Leu Gln Lys His
                                                 45
        35
                             40
Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
                                             60
                         55
    50
Lys Ser Phe Ser Arg Ser Asp His Leu Gln Arg His Gln Arg Thr His
```

70

65

75

Thr Gly Glu Lys

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<210>
      31
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      10
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<223> Degenerate DNA probe
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      (7)..(10)
      Nucleotides 7-10 are "n" wherein "n" = g, a, t, or c.
<223>
<400> 31
ggggaannnn
                                                                     10
<210>
      32
<211>
      26
<212> DNA
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      Zinc finger domain target sequence
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<221> misc_feature
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      (14)..(16)
<223> Nucleotides 14-16 are "n" wherein "n" = g, a, t, or c.
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tatatatagg ggaannngta tatata
26
<210>
      33
      26
<211>
<212> DNA
<213> Artificial Sequence
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      Zinc finger domain target sequence
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      (15)..(17)
<222>
<223> Nucleotides 15-17 are "n" wherein "n" = g, a, t, or c.
```

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<400> 33
tatatatagg ggaannnata tatata
                                                                      26
<210>
      34
<211>
      26
<212> DNA
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     Zinc finger domain target sequence
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<221> misc feature
<222> (15)..(17)
<223> Nucleotides 15-17 are "n" wherein "n" = g, a, t, or c.
<400> 34
tatatatagg ggaannntta tatata
                                                                      26
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      35
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<221> misc_feature
      (15)..(17)
<223> Nucleotides 15-17 are "n" wherein "n" = g, a, t, or c.
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                                                                      26
tatatatagg ggaannncta tatata
<210>
       36
<211>
       60
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<223> Partial zinc finger domain oligomer
<220>
<221> misc feature
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      (45)..(56)
<223> Nucleotides 45-47 and 51-56 are "n" wherein "n" = g, a, t, or c.
```

- 113 -

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<400> 36
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                                                                     60
<210> 37
<211> 60
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      DNA
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      Partial zinc finger domain oligomer
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<221> misc feature
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      (37)..(51)
<223> Nucleotides 37-39 and 46-51 are "n" wherein "n" = g, a, t, or c.
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tttgtatggt ttttcaccgg tatgggtacg ctgatgnnnc tgcaannnnn ngctnnngct
                                                                     60
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<223> Partial zinc finger domain oligomer
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       (46)..(57)
       Nucleotides 46-48 and 52-57 are "n" wherein "n" = g, a, t, or c.
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ggtgaaaaac catacaaatg tccagagtgc ggcaaatctt tctctnnntc tnnnnnctt
                                                                     60
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       39
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<220>
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       misc feature
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       (37)..(51)
<223> Nucleotides 37-39 and 46-51 are "n" wherein "n" = g, a, t, or c.
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<210> 40
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<223> Partial zinc finger domain oligomer
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<222> (48)..(58)
<223> Nucleotides 48-50 and 54-58 are "n" wherein "n" = g, a, t, or c.
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                                                                      56
<210> 41
<211> 55
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<223> Partial zinc finger domain oligomer
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<221> misc feature
<222> (28)..(48)
<223> Nucleotides 28-30, 37-42 and 46-48 are "n" wherein "n" =
 g, a, t, or c
<400> 41
cttctcccc gtgtgcgtgc gttggtgnnn ttgtaannnn nnactnnnac taaag
                                                                      55
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gggcccggtc tcgaattcgg ggagaagccg tataaatgtc cggaa
                                                                      45
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cttgtaaggc ttctcgccag tgtgagtacg ctgatgnnnc tgaagnnnnn nagannnaga

60

<400> 39

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cccgggggtc tcaagctttt acttctcccc cgtgtgcgtg cgttggtg
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<223> Amino acid 15 is "Xaa" wherein "Xaa" = Z2 wherein Z2 = Ser, Asn,
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<223> Amino acid 16 is "Xaa" wherein "Xaa" = Z3 wherein Z3 = His, Asn,
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<220>
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Leu Gln Xaa His Gln Arg Thr His Thr Gly Glu Lys
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